Forensic strategy for the detection of plant toxin ricin

YASUO SETO, MIEKO KANAMORI-KATAOKA, ISAAC OHSAWA, KOUICHIRO TSUGE, TAKESHI OHMORI, YASUO TAKAYAMA and RYOJI SEKIOKAS

National Research Institute of Police Science, 6-3-1, Kashiwanoha, 277-0882, Kashiwa, Japan

AIMS: Considering the recent situations involving chemical and biological terrorism, the threat of terrorism using proteinous toxins is now emerging, and so, it is necessary to establish the analytical methods for determining proteinous toxins. Ricin, which is produced in the caster bean, is a heterodimer where ribotoxin (RTA) is linked with a galactose-binding lectin (RTB) via a disulfide bond. Our laboratory established both the on-site detection and laboratory analysis system for ricin in the standpoint of homeland security and forensics, and here introduce our results.

METHODS: Ricin was provided from the Honen Corporation, and used under the permission of the Minister of Economy, Trade and Industry. Bio Threat AlertTM (BTA) Test Strips for "Ricin" were obtained from Tetracore LLC. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) was performed using 10% gel and 2-mercaptoethanol reduction. Matrix-assisted laser desorption ionization-time of flight-mass spectrometry (MALDI-TOF-MS) was performed with linear mode and positive polarity with matrix of sinapinic acid in acetonitrile-water. Ricin was denatured with guanidine-dithiothreithol-iodoacetic acid, digested by trypsin, and analyzed by liquid chromatography-mass spectrometry (LC-MS) with ricin, 2 protein bands (60 kDa) were observed by non-reduced SDS-PAGE, and from reduced authentic ricin, several protein bands (30 kDa) were observed. Two distinct peaks, singly (m/z 62,592) and doubly (m/z 31,401) protonated molecular ions were detected on the mass spectrum of authentic ricin by MALDI-TOF-MS, and two partly-overlapped peaks around m/z 31,000-32,000 were observed for reduced authentic ricin. The digested ricin gave multiple peptide peaks, and ESI mass spectra were obtained from their peaks, giving singly and doubly protonated molecular ions. From the MS/MS analysis, clear peptidic fragmentation patterns were obtained, leading to peptide sequencing.

CONCLUSION: The BTA system provides rapid and easy on-site detection of ricin in evidenced samples such as white powder. After being brought to the forensic laboratory, SDS-PAGE and MALDI-TOF-MS provide screening of ricin in evidence samples without authetic ricin, and digestion LC-MS provides absolute confirmation of ricin.

KEYWORDS: *Toxin, Detection, Identification, Terrorism*

Corresponding author: seto@nrips.go.jp